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Original Article

Histological features of the uterine tubes following administration of aqueous tobacco extract in female albino rats

Omondi D.O., Kigera J., Pulei A., Munguti J.

ABSTRACT

Background and aim: Tobacco consumption among women of reproductive age is associated with more than twofold increase in the risk of tubal ectopic pregnancy. Established adverse effects of tobacco on reproduction include infertility, low birth weight and miscarriages. This study aimed at investigating the effects of liquid tobacco extract on the mucosa of the uterine tubes using rat models.

Materials and Methods: Thirty rats (6 baseline, 12 experimental and 12 control) were used in the study. The baseline rats were sacrificed at the start of the experiment. The rats in the experimental group received 30mg/kg body weight of the extract while the rats in the control group received normal saline. On days 15 and 30, 6 rats from the control group and 6 from the experimental group were respectively euthanized, perfused and their uterine tubes harvested for processing and embedding in paraffin wax. Photomicrographs were taken and analyzed.

Results: A decrease in the mucosal thickness, height and number of the mucosal folds was observed in the experimental group. A squamous transformation of the normal columnar epithelium of the uterine tubes was also observed.

Conclusion: These microscopic alterations may underlie the functional impairments of the uterine tube associated with tobacco consumption (whether by smoking or chewing), increasing the risk of ectopic pregnancy and infertility.

Keywords:
Tobacco consumption; Uterine tubes; Histological features; Infertility

INTRODUCTION

The uterine tubes (UT) are muscular conduits that connect the ovaries to the uterus and are divided into four regions: the fimbrial end, infundibulum, ampulla, and isthmus. Histologically, the uterine tube consists of three layers: an external serosal layer, an intermediate muscular layer, and an internal mucosal layer (Pawlina and Ross, 2018). The primary functions of the UT include capturing ovulated oocyte-cumulus complexes, transporting them to the ampulla for fertilization, and subsequently conveying the preimplantation embryo to the uterus at a precisely regulated rate for implantation (Talbot and Riveles, 2005). Additionally, sperm capacitation also occurs within the UT (Hunter and Rodriguez-Martinez, 2004).

Globally, the prevalence of tobacco smoking among women is approximately 7.5%, with notably higher rates in developing countries (Sreeramareddy et al., 2014; Iqbal et al., 2015).

In Kenya, the Global Adult Tobacco Survey of 2014 (Ministry of Health, 2014) reported that 4.5% of women consume tobacco in both smoking and smokeless forms. Our study focuses on the latter mode of consumption, specifically the effects of tobacco extract.

Tobacco consumption has been shown to impair various aspects of female reproductive function, including folliculogenesis, steroidogenesis, embryo transport, endometrial receptivity, and endometrial angiogenesis (Dechanet *et al*., 2010). Tobacco smoking, in particular, is a major risk factor for tubal ectopic pregnancy (EP), with more than half of smokers at risk (Handler *et al*., 1989; Horne *et al*., 2014). Exposure to cigarette smoke has been linked to deficiencies in oocyte retrieval, reduced ciliary beat frequency, and excessive adhesion between oocytes and epithelial cells (Dechanet *et al*., 2010). Previous studies have focused primarily on the functional impairments caused by tobacco on fertility, such as reduced gamete transport and increased risk of ectopic pregnancies. However, limited research has explored the histological alterations in the uterine tube that underlie these functional disruptions.

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While existing studies have examined nicotine's effects on other reproductive structures, including the uterus and ovaries (Iranloye and Bolarinwa, 2009; Patil *et al*., 1998), data on the specific structural changes in the uterine tube mucosa following tobacco exposure remain scarce. Understanding these histological modifications is critical to explaining the mechanistic basis of tobacco-induced infertility and ectopic pregnancies.

This study aimed to fill this gap by evaluating the effects of aqueous tobacco extract on the uterine tube mucosa in a rat model, simulating the chewing mode of tobacco intake. By providing quantitative histological evidence, this research sought to enhance understanding of how tobacco exposure alters the structural integrity of the uterine tube and contributes to reproductive dysfunction.

MATERIALS AND METHODS

Sample size, study design and setting

Rats used in this study were obtained from the Department of Zoology Animal House at the University of Nairobi. Animal care and drug administration were conducted at the same facility, while histological processing of the uterine tubes was carried out at the *Department of Human Anatomy and Physiology*, University of Nairobi.

Thirty female rats weighing between 180–230 g and aged two months were used in this study. By postnatal day 60 (two months), most behavioral, physiological, and reproductive systems in rats reach full maturation, and they are considered adults beyond this age. Rats displaying any visible pathology were excluded, with assessments conducted through observation and assistance from the animal house attendants.

Approval for the study was obtained from the Biosafety, Animal Use, and Ethics Committee, Faculty of Veterinary Medicine, University of Nairobi (REF: FVM BAUEC/2019/205). The study adhered to the guidelines provided by the committee, ensuring compliance with ethical principles outlined in the Animal Research: Reporting of In Vivo Experiments (ARRIVE Guidelines) (Kilkenny *et al*., 2010).

The experimental animals were housed in rat cages measuring 109 cm × 69 cm × 77.5 cm. The cage floors were lined with wooden shavings, which were replaced every two days following thorough cleaning. Before the start of the study, the rats were acclimatized for three days in their cages and then transferred to the study area, maintained under a 12-hour light/dark diurnal cycle. Standard pellets and water were provided *ad libitum*.

To prevent injury, the rats were handled with bite-resistant gloves. During transfer, feeding, or cleaning, they were held at the base of their tails using the index finger and thumb of the non-dominant hand. Protective equipment—including a clean lab coat, sterile latex gloves, a gas mask, and goggles—was worn during tissue fixation and processing to minimize exposure to skin and eye irritants, as well as carcinogenic reagents such as formaldehyde. Tissue processing was conducted in a fume cupboard within a well-ventilated laboratory at the Department of Human Anatomy, University of Nairobi.

Preparation of the Tobacco extract

Dried tobacco leaves (*Nicotiana tabacum*) were purchased from a single local supplier in Bungoma, Western Kenya, a region known for tobacco farming. A single supplier was chosen to minimize variability and enhance the reproducibility of results. To further ensure consistency, all tobacco leaves were harvested at the same time.

Tobacco extraction was conducted in the Department of Biochemistry following the protocol described by Gambo *et al*. (2013). Two hundred grams of dried tobacco leaves were boiled in 2000 mL of distilled water for one hour. The resulting solution was filtered using filter paper to remove solid residues, then centrifuged at 4000 revolutions per minute for 15 minutes in a standard laboratory centrifuge. The supernatant was carefully poured into a clean glass jar and later freeze-dried using a freeze-drying machine in the endocrinology laboratory, yielding the extract in powdered form.

Animal intervention

Six rats were randomly selected and sacrificed on the first day of the experiment to establish the baseline histomorphology of the uterine tube. The remaining 24 rats were randomly divided into two equal groups: a control group and an experimental group. The experimental group received a daily dose of 30 mg/kg body weight of tobacco extract dissolved in 0.5 mL of distilled water. This dosage was selected based on the study by (Bello *et al*., 2013). The control group received 0.5 mL of normal saline.

Both groups were administered their respective treatments via oral gavage. On days 15 and 30, six rats from each group were euthanized, perfused, and their uterine tubes harvested for processing and embedding in paraffin wax.

Tissue harvesting, Processing and staining

The rats were weighed and then euthanized by placing them in sealed containers with 1% halothane-soaked cotton wool. Euthanasia was confirmed by the absence of a heartbeat and the loss of the blink reflex. The rats were then perfused via the left ventricle, first with normal saline to flush out blood, followed by 10% formal saline to fix the body organs. The sternum was removed to allow direct access to the heart during perfusion.

The uterine tubes were harvested through a midline abdominal incision extending to the pelvic region, with the skin flaps reflected laterally. The urinary bladder, along with some coils of the intestine, was carefully reflected to expose the uterus and uterine tubes. To ensure proper orientation of the tubes during blocking and sectioning, the ovaries were removed together with the uterine tubes using a pair of forceps and a sharp blade. The harvested uterine tubes were immediately placed in 10% formalin within specimen bottles and fixed for 24 hours before routine processing and staining for light microscopy.

The fixed uterine tubes underwent dehydration in ascending grades of ethyl alcohol, starting at 70% and progressing to absolute alcohol at one-hour intervals. The tissues were then placed in a 1:1 alcohol-toluene mixture, cleared in toluene for two hours, and impregnated with paraffin wax at 58ºC for 12 hours. They were then embedded in paraffin wax and allowed to cool before being mounted on wooden blocks for sectioning. Using a microtome, the tissues were sectioned into 7-micrometer-thick slices. The sections were floated in a warm water bath and mounted onto clean glass slides before being dried in an oven at 38ºC for 12 hours.

To prepare the slides for staining, the dried sections were first immersed in xylene to remove the paraffin wax, followed by rehydration through descending grades of ethanol (100%, 95%, and 70%). The sections were then stained with Ehrlich's Hematoxylin for 15 minutes and washed in running water for another 15 minutes to remove excess stain. They were counterstained with 1% Eosin solution for three minutes, followed by dehydration in ascending grades of ethanol from 70% to absolute alcohol. Finally, the sections were cleared in two changes of xylene before mounting.

Morphometric Analysis

Photomicrographs were captured using a Zeiss™ digital photomicroscope (Carl Zeiss AG, Oberkochen, Germany) for histomorphometric analysis. Image analysis was performed using Fiji ImageJ software. The quantitative parameters measured included the number and height of mucosal folds and the thicknesses of the mucosal layer.

The collected data were entered into the Statistical Package for Social Sciences (SPSS) software (version 22.0, Chicago, Illinois) for coding, tabulation, and statistical analysis. Means and standard deviations were computed for all variables.

RESULTS

Thirty female rats, aged between two and three months, were used in this study. At the start of the experiment, the average weight of the control group was 229.8 ± 16.19g, while that of the experimental group was 235.83 ± 18.04g. By day 30, the control group showed an increase in weight, reaching 245.8 ± 30.13g, whereas the experimental group experienced a decline, with an average weight of 205.83 ± 20.92g.

At baseline (day 0), the rat uterine tube (UT) was composed of three distinct histological layers: mucosa, muscular, and serosal layers (Figure 1). The mucosal layer consisted of a simple columnar epithelium resting on a cellular lamina propria (Figure 1), and it was highly folded, with projections extending into the lumen, creating an irregular surface. The muscular layer was composed of concentric layers of smooth muscle cells, while the serosal layer consisted of blood vessels and connective tissue interspersed with cells. This structural organization was observed in all baseline samples and remained consistent in the control group throughout the experiment.

In contrast, significant histological changes were observed in the experimental group. By day 15, the epithelial cells of the mucosa had undergone a noticeable reduction in height, losing their characteristic columnar appearance. The lamina propria exhibited a decline in cellular density, while the mucosal folds were both fewer in number and shorter in height. This resulted in an increase in the distance between successive folds. By day 30, these changes had become even more pronounced. The epithelial cells showed further reduction in height, and the mucosal folds continued to diminish in both height and number, leading to a marked increase in the spacing between them (Figure 2). Additionally, an increase in vascularization was observed, with some blood vessels appearing dilated and enlarged (Figure 2).

Quantitative analysis confirmed these observations. The thickness of the mucosa was nearly identical in both groups at day 15, with the experimental group measuring 18.21µm ± 7.38 and the control group 18.33µm ± 3.51 (Figure 3). However, by day 30, the mucosal thickness in the experimental group had decreased to 12.73µm ± 3.12, while the control group maintained a relatively stable measurement of 18.06µm ± 5.81.

Similarly, there was a progressive decline in the number of mucosal folds throughout the study period in both groups, though the reduction was more pronounced in the experimental group (Figure 4). At day 15, the experimental group had an average of six mucosal folds (6 ± 3.40), whereas the control group had thirteen (13 ± 2.56). By day 30, the number of folds had further decreased to five (5 ± 2.99) in the experimental group, while the control group retained an average of eleven folds (11 ± 3.91).

A similar trend was observed in the height of the mucosal folds (Figure 5). By day 15, the experimental group exhibited a significantly lower mean mucosal fold height of 56.35µm ± 27.03, compared to 124.76µm ± 37.44 in the control group. This decline continued, and by day 30, the mean height of the mucosal folds in the experimental group had further decreased to 43.84µm ± 24.27, while the control group measured 112.85µm ± 51.76.

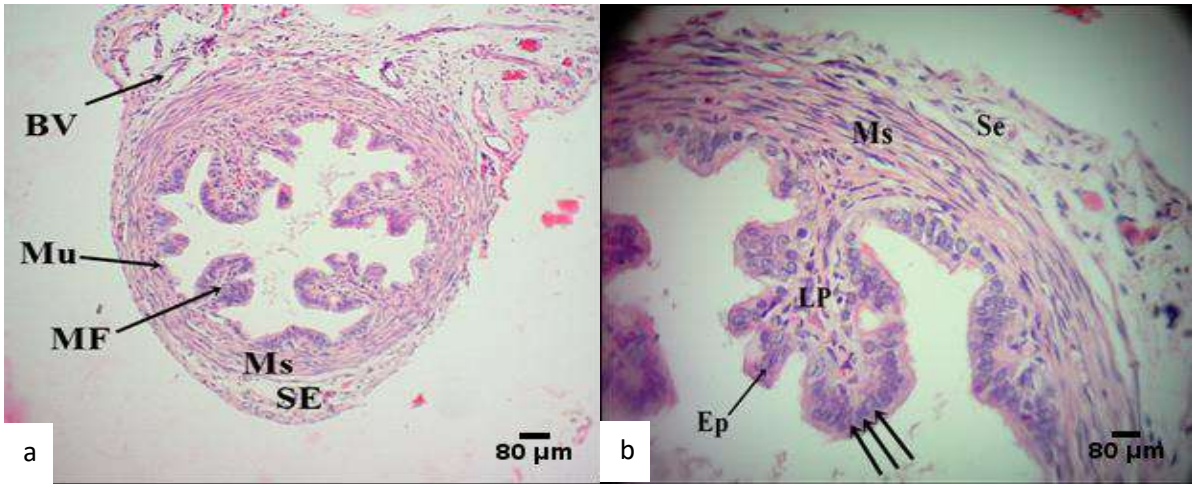

Figure 1: Photomicrographs showing the light microscopic features of the rat uterine tube at baseline. Notice that the tube is made up of three layers; the mucosa (Mu), the muscular layer (Ms) and the serosa (Se). The mucosa is thrown into folds (MF) that project into the lumen of the tube making it irregular. Also notice the presence of a blood vessel (BV) in the serosal layer. (Stain: Hematoxylin and Eosin, H&E; magnification= x100) **(b)** A higher magnification of the uterine tube showing the mucosa organized into an epithelium (Ep) overlying a highly cellular lamina propria (LP). The epithelium is made up of simple columnar cells (arrows). (H&E; 400×)

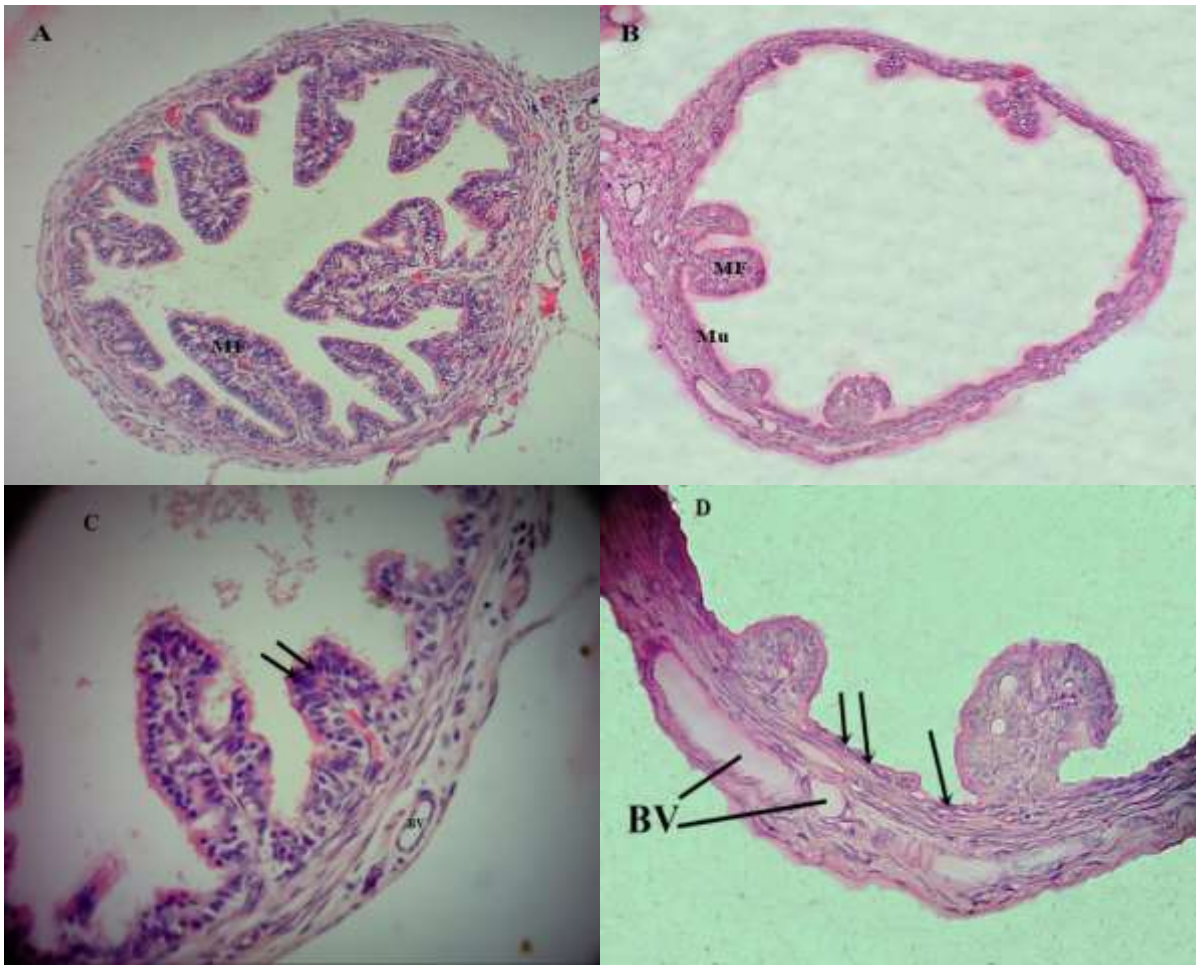

Figure 2: Photomicrographs showing the general morphological changes of the uterine tubes. **(A)** Photomicrograph of the uterine tube of control group showing the uterine tube having closely packed mucosal folds (MF). Note the height of the mucosal folds. H & E ×100. **(B)** Photomicrograph of the experimental group showing a marked reduction in the number and height of the MF. Note the resultant increase in the distance between successive folds. Mu- mucosa. H & E ×100. **(C)** A higher magnification of **'A'** showing the simple columnar cells of the epithelium (arrows). Note the size of the blood vessels (BV). H & E ×400. **(D)** A higher magnification of **'B'** showing squamous epithelium (arrows). Notice that the caliber of some blood vessels (BV) appears to have increased. H&E; 400×.

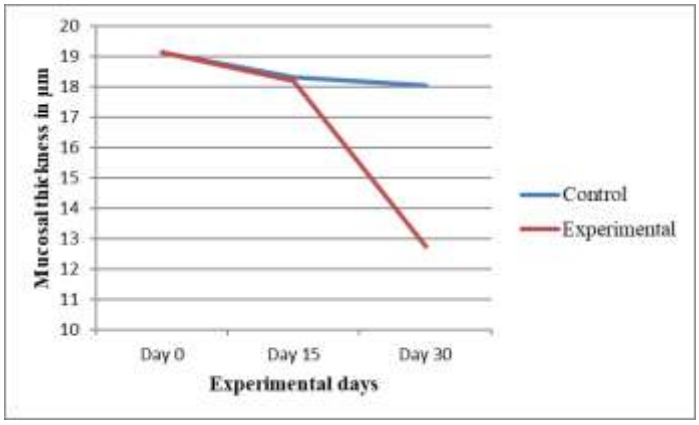

Figure 3: Line graph showing the mean thickness of the mucosa over time between the experimental and the control groups.

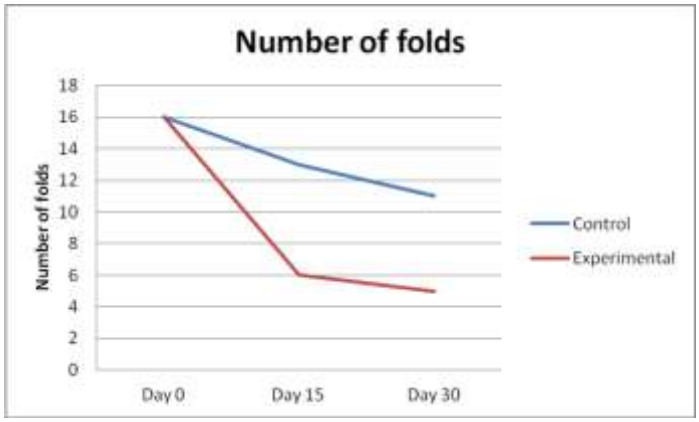

Figure 4: Line graph showing mean number of mucosal folds over time between the experimental and control groups.

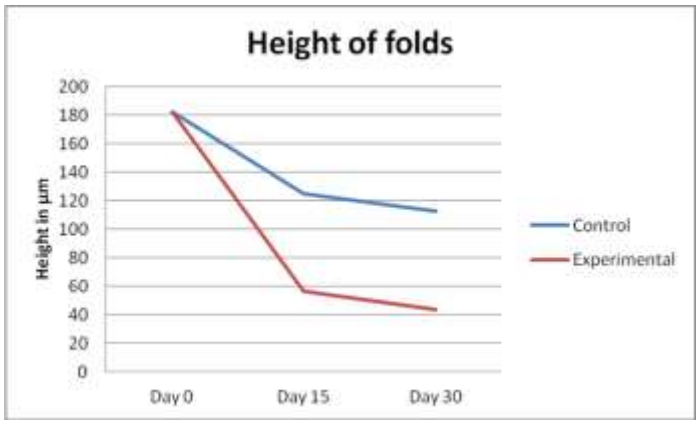

Figure 5: Line graph showing the mean height of mucosal folds over time between the experimental and the control groups.

DISCUSSION

Tobacco administration in rats led to significant histological changes in the uterine tube (UT), particularly a decrease in epithelial height, which caused the epithelium to appear more squamous. This reduction in epithelial height contributed to an overall decrease in mucosal thickness. Additionally, increased vascularization and an enlargement of certain blood vessels were observed following tobacco extract administration. These findings align with those of (Iranloye and Bolarinwa, 2009), who reported endometrial degeneration in rats exposed to nicotine, as well as (Halder *et al*., 2015), who noted a decrease in luminal epithelial height in the uterus after nicotine exposure. A similar pattern has been observed in airway epithelium, where cigarette smoke exposure disrupted tight junctions, leading to epithelial barrier dysfunction (Aghapour *et al*., 2017).

Moreover, tobacco exposure significantly reduced both the number and height of mucosal folds in the UT. This atrophic effect on the mucosal folds is reminiscent of intestinal villi shrinkage observed in male rats exposed to cigarette smoke (Zuo *et al*., 2014). The observed changes in the UT may be attributed to tissue hypoxia, which can result in cell death. This hypothesis is consistent with previous research showing that nicotine induces tissue hypoxia (Zhang *et al*., 2007). Another potential mechanism for these histological changes is the damaging effect of hydroxyl free radicals. Nicotine administration has been linked to an increase in free fatty acids, which subsequently undergo peroxidation, generating harmful free radicals (Yildiz, 2004). Additionally, nicotine has been shown to reduce the activity of free radical-scavenging enzymes (Ashakumary and Vijayammal, 1996), further exacerbating oxidative damage.

These structural alterations in the UT epithelium may help explain the increased risk of infertility and ectopic pregnancy (EP) associated with cigarette smoke exposure. The UT epithelium plays a critical role in sperm capacitation (Hunter and Rodriguez-Martinez, 2004) and early embryo development (Leese *et al*., 2008). It secretes essential fluids that provide nutrition to the developing conceptus (Leese *et al*., 2008). A reduction in epithelial height may compromise this secretory function, potentially impairing early embryonic development. Furthermore, disruptions in epithelial integrity could interfere with sperm capacitation and fertilization, contributing to infertility associated with smoking.

The observed decrease in the number and height of mucosal folds may also have implications for tubal embryo transport, thereby increasing the risk of EP. Mucosal folds enhance the surface area for interaction between the preimplantation embryo and the UT epithelium (Binelli *et al*., 2018). This interaction is crucial for the efficient transport of the embryo to the uterus for implantation (Dechanet *et al*., 2010). A reduction in mucosal fold surface area could disrupt this process, potentially leading to abnormal implantation and an increased incidence of EP.

Overall, these findings provide further evidence of the deleterious effects of tobacco exposure on reproductive health. The structural changes observed in the UT may contribute to infertility and ectopic pregnancy, reinforcing the need for increased awareness of the reproductive risks associated with smoking.

Conclusion: The findings from this study demonstrate that even when tobacco is consumed through chewing rather than smoking, its detrimental effects on reproductive health remain evident. The structural alterations observed in the uterine tube, including reduced epithelial height, decreased mucosal thickness,

and diminished mucosal folds, closely resemble the damage reported in studies on nicotine exposure through smoking. These changes may impair sperm capacitation, early embryo development, and embryo transport, thereby increasing the risk of infertility and ectopic pregnancy. The similarities between the effects of chewing tobacco and smoking suggest that nicotine and other harmful compounds in tobacco exert their toxic effects on the reproductive system regardless of the route of administration. This underscores the need for heightened awareness about the reproductive risks associated with all forms of tobacco consumption, including chewing, which may be perceived as a less harmful alternative to smoking.

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